Sesquiterpene Lactones of Sagebrush. New Guaianolides from *Artemisia cana* ssp. *viscidula*[1]

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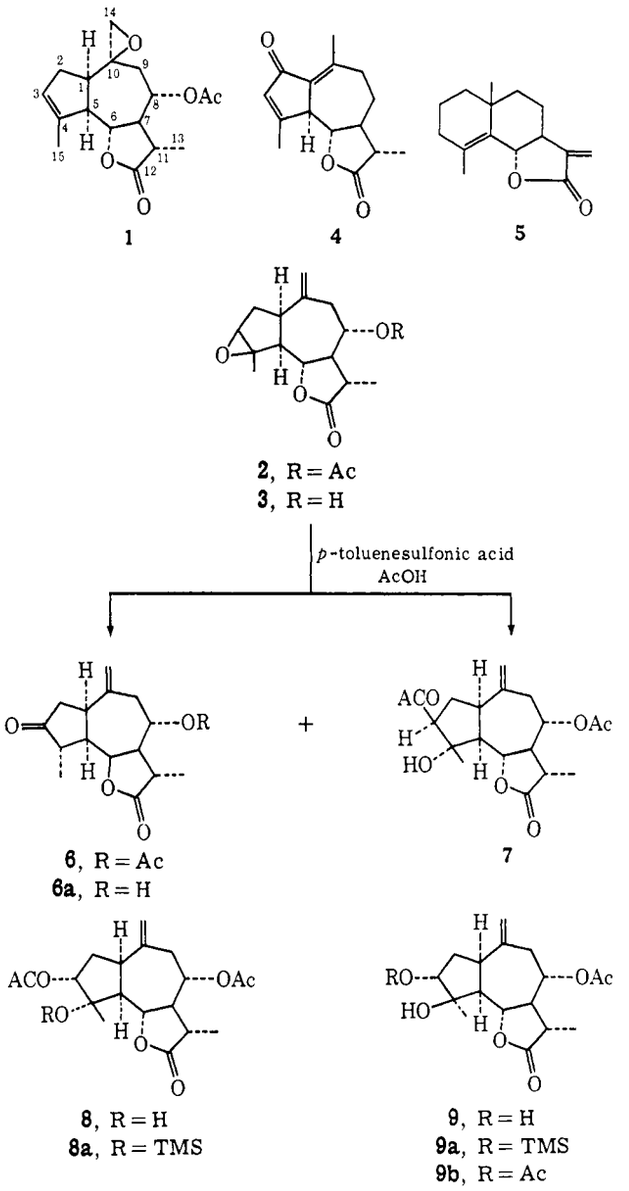
Four crystalline sesquiterpene lactones were isolated from samples of *Artemisia cana* Pursh ssp. *viscidula* (Osterhout) Beetle collected in Montana. Three of these lactones were found to be new guaianolides and were named viscidulin A (**1**), B (**2**) and C (**3**). The other compound was shown to be the known guaianolide deacetoxymatricarin (**4**). Structures of the new compounds were determined by their spectral properties and chemical reactions. Viscidulin B was related to known guaianolides by synthesis from cumambrin B (**10**) and conversion to isoamberboin (**6a**). The new guaianolides were correlated by acetylation of viscidulin C to viscidulin B and epoxidation of viscidulin B and viscidulin A to a common diepoxide (**18**).

The sesquiterpene lactones of *Artemisia cana* Pursh ssp. *viscidula* (Osterhout) Beetle were investigated as a part of this laboratory's program on chemical constituents of sagebrush in Montana.[3-6] Tlc of a sample of this subspecies (denoted by **ACV1**) collected near Eureka Basin, Mont., in August 1970, showed four distinct spots. One of these spots (second from top) corresponded with the known guaianolide deacetoxymatricarin (**4**). The other spots represented new guaianolides named viscidulin A (**1**), B (**2**), and C (**3**). Deacetoxymatricarin was absent in a sample (**ACV2**) collected the next August at Beaver Creek, Snowline Ranch, another Montana location. None of these samples, however, showed the presence of arbusculin B (**5**), reported for a sample of this subspecies collected in Wyoming.[7] These compounds were isolated from the plant materials by the established methods for separation of sesquiterpene lactones, and their structures were determined through spectroscopic studies and correlation with the known guaianolides isoamberboin[8] (**6a**) and cumambrin B (**10**).[9,10] See Chart I.

CHART I



Results and Discussion

Viscidulin B (**2**).—Elemental analysis and mass spectrum of viscidulin B showed the empirical formula of $C_{17}H_{22}O_5$ and a molecular weight of 306. The compound showed an ir band at 1779 cm^{-1} and a moderate uv end absorption indicative of a γ-lactone moiety. Further ir bands at 1735 and 1254 cm^{-1} coupled with C_{17} composition of the sesquiterpene lactone and mass spectral fragmentation peaks at *m/e* 264 (M − 42) and 246 (M − 60) suggested the presence of an acetate group. Aromatization of the molecule as discussed later gave chamazulene, showing the guaianolide carbon skeleton. Other features of the ir spectrum included a weak band at 1647 cm^{-1} and a medium band at 885 cm^{-1}, which suggested the presence of unsaturation. The lactone and the acetate groups accounted for four of the five oxygen atoms in the molecule, and the absence of any bands for hydroxyl or keto group in the ir and uv spectra suggested that the fifth oxygen is involved in the formation of a heterocyclic ring. This

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conclusion was supported by a positive epoxide test.[11,12]

Presence of the above functions was confirmed by the nmr spectrum, which also indicated other structural features of the molecule. The C-6 lactone proton appeared as a triplet at 4.08 ppm (J = 10 Hz). The coupling of this proton indicated its trans-diaxial disposition to the hydrogens at C-5 and C-7.[13] A three-proton singlet at 2.03 ppm confirmed the presence of the acetate group, and the unsaturation of the molecule was signaled by two broad one-proton singlets at 4.88 and 5.03 ppm ($W_{1/2}$ = 4 Hz, characteristic of unconjugated *exo*-methylene protons).[5,14] The latter signals assigned to C-10 methylene protons were superimposed on a broad signal for the proton under the acetate group at C-8. Using benzene-d_6 or pyridine-d_5 as a solvent did not help to separate these signals. The C-3 proton of the C-3–C-4 epoxide function appeared as a singlet at 3.33 ppm. The position and nature of this signal was similar to the C-3 protons in cumambrin A epoxide[9] and arteglasin A.[13] A sharp three-proton singlet at 1.52 ppm and a three-proton doublet at 1.15 ppm (J = 6.5 Hz) were assigned to a methyl group attached to the C-4 of the epoxide ring and the C-11 secondary methyl group of the guaianolide structure, respectively. The first signal corresponded closely to the singlets for the C-4 CH_3 groups in cumambrin A epoxide[9] and arteglasin A[13] at 1.60 and 1.64 ppm, respectively. Assuming that the C-11 secondary methyl group is α oriented, as in many lactones of this genus like santonin and deacetoxymatricarin,[15] these data led to the structure **2** for viscidulin B.

Synthesis of Viscidulin B (2).—The principal features of structure **2** were confirmed by synthesis of viscidulin B from the known guaianolide, cumambrin B,[9,10] that was isolated in good yield from a Montana sample of *A. nova* Nels. This lactone (**10**) was acetylated to give the monoacetate cumambrin A (**11**) (Chart II). Controlled hydrogenation of cumambrin A gave crystalline dihydrocumambrin A[9] (**12**), which was dehydrated with pyridine and thionyl chloride. Tlc of the gummy product gave a single spot, but the nmr spectrum showed that it is a mixture of unsaturated lactones. Since the desired compound **13** could not be obtained in pure form, the mixture was used as such for the next reaction. Epoxidation of this gum with 1 mol equiv of *m*-chloroperbenzoic acid gave another complex mixture from which viscidulin B (**2**) was isolated as a crystalline product after extensive column chromatography.

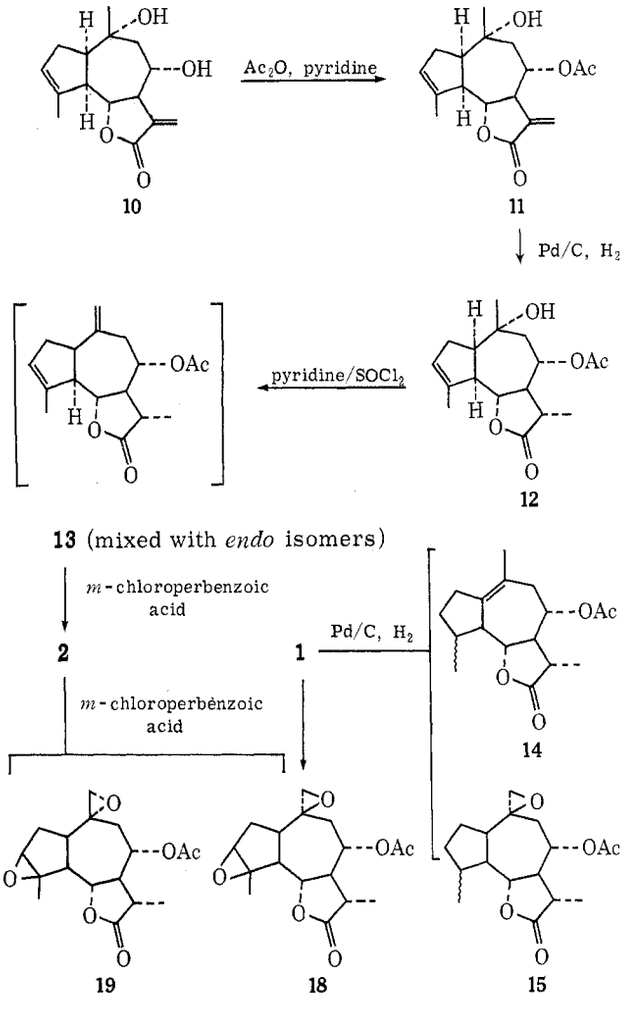
Viscidulin B Derivatives.—The above synthesis does not establish the stereochemistry of the C-11 methyl and the C-3–C-4 epoxide groups. The α orientation of the methyl group was confirmed by conversion of viscidulin B (**2**) to the known guaianolide isoamberboin (**6a**)[8] through the opening of the epoxide ring.

Initial attempts for the opening of the epoxide ring by hydrogenation or treatment with acetic anhydride and *p*-toluenesulfonic acid[16] gave intractable mixtures. A dark-colored oil produced by the latter treatment, however, after chromatography on silica gel gave traces of a blue oil which presumably was an azulene. Finally, treatment of viscidulin B with *p*-toluenesulfonic acid in glacial acetic acid resulted in the opening of the epoxide function and gave a reaction mixture showing four major spots on tlc. Elaborate column chromatography of the mixture resulted in the separation of four components (**6–9**), three of which (**6**, **8**, and **9**) were obtained in crystalline form. Overheating of compound **7**, which could not be crystallized, gave chamazulene as a blue oil in sufficient quantity to be characterized as the crystalline trinitrobenzene adduct.[10,17]

The chemical structures of lactones **6–9** were determined through the following considerations.

Lactone **6** had the same empirical formula, $C_{17}H_{22}O_5$, as the parent compound and gave mass spectrum peaks at *m/e* 306 (M^+), 264 (M − 42), 246 (M − 60), and 218 (M − 60 − 28). Its ir spectrum showed γ-lactone (1769 cm^{-1}), cyclopentanone (1725 cm^{-1}), and acetate (1725, 1220 cm^{-1}) bands. The presence of the cyclopentanone ring was also confirmed by the uv absorp-

CHART II



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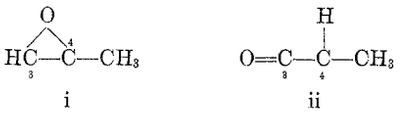
tion at 294 nm (ϵ 24.14). Furthermore, the nmr data listed in Table I showed that the singlet at 3.33 ppm due to C-3 H in the parent compound (**2**) has disappeared and the singlet at 1.52 ppm due to C-4 CH_3 in the parent compound has been replaced by a doublet at 1.27 ppm (J = 6.5 Hz) as the only discernible changes after the reaction. These changes indicated that



i ii

group i in the original compound has been converted to ii in lactone **6**. Deacetylation of this lactone gave the known guaianolide isoamberboin (**6a**),[8] confirming the spectroscopic structural deductions and the α orientation of the C-11 methyl group.

Lactone **7** could not be obtained in crystalline form, but it was purified chromatographically to give sharp ir and nmr spectra. The ir spectrum showed the presence of a hydroxyl group (3333 cm^{-1}) and unsaturation (1639 cm^{-1}) functions. In the nmr spectrum, the hydroxyl proton appeared as a broad singlet at 3.36 ppm which exchanged with D_2O. There were also two three-proton singlets at 2.07 and 1.97 ppm, indicating the presence of an additional acetate group. The new acetate group was evidently located at C-3 and the hydroxyl group at C-4 because the C-3 H gave a narrow triplet at 4.68 ppm (J = 5 Hz) instead of a singlet at 3.33 ppm in the parent lactone (**2**) and the C-4 CH_3 signal remained almost unchanged at 1.6 ppm. Other features of the nmr spectrum were closely similar to those of the starting material. These data indicated the diol–monoacetate opening of the epoxide group shown in structure **7**. Presence of an additional acetate group was consistent with peaks at m/e 306 (M − 60), 246 (M − 60 − 60), and 231 (M − 60 − 60 − 15).

Lactone **8** was obtained as a crystalline compound. It had the empirical formula of $C_{19}H_{26}O_7$ and the same ir bands as lactone **7**. It formed a monotrimethylsilyl derivative in which the hydroxyl band had disappeared. Attempted acetylation under normal conditions failed, indicating that the hydroxyl group must be tertiary. Presence of two acetate groups was suggested by the mass spectrum with peaks at m/e 366 (M^+), 324 (M − 42), 306 (M − 60), 264 (M − 60 − 42), and 246 (M − 60 − 60). These observations along with the nmr data (see Table I) indicated that lactone **8** is the C-3 epimer of lactone **7**. The configuration of C-3 and C-4 substituents of these compounds was determined by the pyridine-induced chemical shift of the C-6 H and C-4 CH_3 nmr signals (Table I).

Lactone **9** gave the elemental analysis of $C_{17}H_{24}O_6$ and showed ir bands for hydroxyl (3483 and 3389 cm^{-1}), γ-lactone (1763 cm^{-1}), acetate (1742 and 1240 cm^{-1}), and unsaturation (892 cm^{-1}) groups. The mass spectrum with peaks at m/e 306 (M − 18), 264 (M − 60), and 246 (M − 60 − 18) also suggested the presence of hydroxyl and acetate groups. The lactone formed monotrimethylsilyl and monoacetyl derivatives under normal conditions. However, both of these compounds still showed ir bands for hydroxyl groups, suggesting the presence of a resistant tertiary hydroxyl group in the lactone. These observations, along with the nmr spectra of the lactone and its derivatives (Table I), supported the structure assigned to lactone **9**, including configuration of C-3 and C-4 substituents determined from the pyridine-induced chemical shifts of C-6 H, C-4 CH_3, and C_3 H.

Viscidulin C (3).—Viscidulin C had the empirical formula of $C_{15}H_{20}O_4$ and gave a parent ion at m/e 264 in the mass spectrum. The ir spectrum showed the presence of hydroxyl (3496 cm^{-1}), γ-lactone (1745 cm^{-1}), and unsaturation (1639, 896 cm^{-1}) functions. The nmr spectral features (Table I) were similar to those of viscidulin B, except for a broad signal which appeared at 3.75 ppm instead of 4.7 to 5.0 ppm and the absence of the acetate singlet at 2.03 ppm. These differences suggested the presence of a hydroxyl group at C-8 instead of the acetate group. This conclusion was confirmed by acetylation of viscidulin C to viscidulin B.

Viscidulin A (1).—Viscidulin A had the empirical formula of $C_{17}H_{22}O_5$, a moderate uv end absorption, and an ir band at 1773 cm^{-1}, suggesting a γ-lactone structure as in viscidulin B and C. Other ir bands at 1733 and 1243 cm^{-1}, the C_{17} composition, and mass spectrum peaks at m/e 264 (M − 42) and 246 (M − 60) suggested the presence of an acetate group. A weak band at 1640 cm^{-1} indicated unsaturation of the molecule. As in **2**, one oxygen atom appeared to form a heterocyclic ring, since no hydroxyl or carbonyl group was detected in the ir and uv spectra and viscidulin A gave a positive test for epoxide function.[11,12]

The nmr spectum of viscidulin A indicated the guaianolide structure **1**. The lactone proton appeared as a triplet at 4.24 ppm (J = 9.5 Hz) and the proton under the acetate group gave a broad signal at 5.20 ppm (coupled with C-7 H and C-9 H_2) as in viscidulin B, indicating *trans*-lactone closure at C-6 and α orientation of the acetate group at C-8.[13] The secondary methyl group at C-11 gave a doublet at 1.27 ppm (J = 6.5 Hz). Comparison of the nmr spectrum of viscidulin A (**1**) with the spectra of viscidulin B (**2**) and C (**3**) showed the presence of an *exo*-epoxide group at C-10–C-14 in **1** instead of the *endo*-epoxide function at C-3–C-4 in **2** and **3**. Conversely, the double bond in **1** appeared at C-3–C-4 instead of C-10–C-14 in **2** and **3**.

Viscidulin A gave a two-proton singlet at 2.64 ppm for an $-OCH_2$ group instead of the one-proton singlet at 3.30 ppm due to the –OC-3 H group in **2** and **3**. Also, there was a narrow one-proton multiplet at 5.47 ppm and a narrow three-proton doublet at 1.87 ppm (J = = 1 Hz) due to a $CH_3C{=}CH$ group instead of the two one-proton broad singlets at ~5.0 ppm due to the C-10 $=CH_2$ group and the three-proton singlet at ~1.50 ppm for the CH_3C-4 O in **2** and **3**. The epoxide proton singlet at 2.64 ppm in viscidulin A corresponded closely to the singlet signal at 2.80 ppm for the $O^{14}CH_2$ in artefransin.[12] Furthermore, the olefinic proton in viscidulin A was coupled with the C-2 protons to give a narrow multiplet ($W_{1/2}$ = 5 Hz) as in cumamabrin B.[9,10] These data are only consistent with the mutual replacement of the epoxide and unsaturation functions in **2** and **3** to form **1**.

Viscidulin A Derivatives.—Hydrogenation of viscidulin A gave a mixture of four products (**14–17**). One of these products was the expected dihydroviscidulin A

TABLE I

NMR DATA FOR VISCIDULIN GUAIANOLIDES AND RELATED COMPOUNDS

Compd	C-3 H	C-4 CH_3	C-6 H	C-8 H	C-10 $=CH_2$ C-10 CH_3	C-11 $=CH_2$ C-11 CH_3	C-14 H_2	Miscellaneous
1[a]	5.47 (nm, $W_{1/2}$ = 5)	1.87 (d, 1)	4.24 (t, 9.5)	5.20 (br)		1.27 (d, 6.5)	2.64 (s)	2.03 (s), OAc
2[a]	3.33 (s)	1.52 (s)	4.08 (t, 10)	(mx, with C-10 $=CH_2$ signals)	5.03, 4.88 (bs, $W_{1/2}$ = 4)	1.15 (d, 6.5)		2.03 (s), OAc
3[a]	3.30 (s)	1.48 (s)	3.95 (t, 9.5)	3.75 (br)	4.98, 4.90 (bs, $W_{1/2}$ = 5)	1.32 (d, 6.5)		2.12 (bs), OH
4[a]	6.18 (nm, $W_{1/2}$ = 4)	2.27 or 2.4	3.60 (t, 10)		2.27 or 2.4	1.23 (d, 6.5)		
6[a]		1.27 (d, 6.5)	4.01 (t, 9.5)	4.93 (br)	5.10, 4.78 (bs, $W_{1/2}$ = 2.5)	1.17 (d, 6.5)		2.05 (s), OAc
7[a]	4.68 (t, 5)	1.60 (s)	4.40 (t, 9.5)	(mx, with C-10 $=CH_2$ signals)	5.13, 5.02 (bs, $W_{1/2}$ = 4)	1.30 (d, 6.5)		2.07 (s), 1.97 (s), OAc 3.37 (bs), OH
7[b]	(mx, with C-10 $=CH_2$ signals)	1.85 (s)	4.50 (t, 9.5)	(mx, with C-10 $=CH_2$ signals)	5.02 (bs, $W_{1/2}$ = 8)	1.27 (d, 6.5)		1.98 (s), 1.93 (s),OAc
8[a]	(mx, with C-10 $=CH_2$ and C-8 H signals)	1.27 (s)	4.17 (t, 9.5)	(mx, with C-10 $=CH_2$ and C-3 H)	5.19, 5.10 (bs, $W_{1/2}$ = 4.5)	1.27 (d, 6.5)		2.07 (s), 2.09 (s), OAc
8[b]	(mx)	1.33 (s)	4.12 (t, 9.5)	(mx)	4.95 (bs, $W_{1/2}$ = 4)	1.25 (d, 6.5)		1.92 (s), 1.87 (s), AOc 5.75 (br), OH
8a[a]	(mx)	1.22 (s)	4.00 (t, 9.5)	(mx)	4.97 (bs, $W_{1/2}$ = 5)	1.15 (d, 6.5)		1.97 (s), strong, OAc 0.00 (s), OTMS
8a[b]	(mx)	1.25 (s)	4.03 (t, 9.5)	(mx)	4.95 (bs, $W_{1/2}$ = 5)	1.14 (d, 6.5)		1.96 (s), 1.92 (s), OAc
9[b]	4.10 (d, 3.4)	1.71 (s)	4.54 (t, 9.5)	(mx, with C-10 $=CH_2$ signals)	5.13, 5.02 (bs, $W_{1/2}$ = 4)	1.27 (d, 6.5)		1.97 (s), OAc 5.55 (bs), OH
9a[a]	3.79 (d, 3.5)	1.41 (s)	4.22 (t, 9.5)	4.78 (br)	5.07 (bs, $W_{1/2}$ = 4)	1.27 (d, 6.5)		2.05 (s), OAc 0.10 (s), OTMS
9a[b]	3.95 (d, 3.5)	1.57 (s)	4.52 (t, 9.5)	4.90 (br)	5.13, 5.03 (bs)	1.28 (d, 6.5)		1.93 (s), OAc
9b[a]	4.96 (d, 3.5)	1.38 (s)	4.21 (t, 9.5)	4.73 (br)	5.06 (bs, $W_{1/2}$ = 5)	1.30 (d, 6.5)		1.98, 1.94 (s), OAc
9b[b]	5.18 (d, 3.5)	1.49 (s)	4.53 (t, 9.5)	(mx)	5.08, 5.0 (bs, $W_{1/2}$ = 4)	1.27 (d, 6.5)		1.96 (s), strong, OAc
10[c]	5.48 (nm, $W_{1/2}$ = 5.5)	1.88 (s)	3.98 (t, 9.5)	3.70 (br)	1.22 (s)	6.12 (dd, 3.5, 1.5) 6.02 (dd, 3.5, 1.5)		
11[c]	5.53 (mx)	1.82 (s)	3.97 (t, 9.5)	5.00 (br)	1.12 (s)	6.03 (d, 3.0) 5.53 (d, 3.0)		4.35 (s), OH 2.12 (s), OAc
12[a]	5.46 (nm, $W_{1/2}$ = 5.5)	1.82 (s)	4.05 (t, 9.5)	5.08 (br)	1.15 (s)	1.21 (d, 7)		2.05 (s), OAc
14[a]		0.87 (d, 6.5)	3.79 (t, 10)	4.87 (br)	1.70 (s)	1.26 (d, 6.5)		2.03 (s), OAc
15[a]		0.87 (d, 6.5)	4.35 (t, 9.5)	5.12 (br)		1.23 (d, 6.5)	2.47 (s)	2.02 (s), OAc
18[a]	3.31 (s)	1.53 (s)	4.13 (t, 9.5)	5.13 (br)		1.25 (d, 6.5)	2.70 (s)	2.02 (s), OAc
19[a]	3.30 (s)	1.52 (s)	4.04 (dd, 10, 9)	5.04 (br)		1.29 (d, 6.5)	2.50 (s)	2.02 (s), OAc

[a] $CHCl_3$-*d* was used as the general solvent for the spectra discussed in the text. [b] Pyridine-d_5 was used as the solvent. [c] DMSO-d_6 was used as the solvent. The nmr spectra were obtained with the Varian HA-60 nmr spectrometer. TMS was used as the general internal standard (lock) and chloroform was used for **8a**[a] and **9a**.[a] Chemical shifts are quoted in δ (parts per million) and the signals are denoted by s, singlet; d, doublet; dd, doublet of doublets; t, triplet; br, broad; bs, broad singlet; nm, narrow multiplet; mx, mixed signals. Figures in parentheses denote coupling constants in Hz. $W_{1/2}$ represents the width of the signal in Hz at the half-height.

(**15**), which was obtained in crystalline form and readily recognized by its spectral properties.

The following consideration indicated that lactone **14**, which was also obtained as a crystalline material, is formed through the saturation of the C-3–C-4 double bond and elimination of the epoxide ring to produce a new double bond at C-1–C-10. This compound gave the elemental analysis of $C_{17}H_{24}O_4$ and mass spectral

peaks at m/e 232 (M − 60) and 217 (M − 60 − 15). Saturation of C-3–C-4 double bond was evident from the absence of a signal for an olefinic proton and the appearance of a new doublet at 0.87 ppm (J = 6.5 Hz) in the nmr spectrum. Elimination of the epoxide group to form a double bond at C-1–C-10 was reflected by the replacement of the two-proton singlet at 2.64 ppm with a three-proton singlet at 1.70 ppm for the CH_3 at C-10. Other nmr features of this lactone were similar to those of the parent compound.

The remaining hydrogenation products could not be obtained in sufficient quantities to allow complete identification. However, their ir spectra suggested the opening of the epoxide function.

The structural relationship between viscidulin A (**1**) and B (**2**) was established by epoxidation of these compounds to a common diepoxy derivative (**18**). The exo double bond in **2** also produced another isomer (**19**). Comparing the nmr data of this isomer (**19**) with the spectra of **1** and **18** showed a downfield position of C-6 H and C-8 H in latter compounds suggesting β orientation of the C-10 O bond of the epoxy groups in **1** and **18**.[18]

The sesquiterpene components of other Montana species of sagebrush and their taxonomical significance will be discussed in subsequent reports.

Experimental Section[19]

Plant Materials.—Two separate samples of *Artemisia cana* Pursh ssp. *viscidula* (Osterhout) Beetle were collected in Montana.[20] One of these samples, denoted by **ACV1**, was collected from Eureka Basin (T. 12 S, R. 4 W, Section 36) in August 1970 and the other, denoted by **ACV2**, was collected from Beaver Creek in the Snowline Ranch (T. 15 S, R. 6 W, NE quarter of Section 31) in August of 1971.

Tlc of ACV1[5,19] gave four distinct spots corresponding to viscidulin A, deacetoxymatricarin, viscidulin B, and viscidulin C. Tlc of **ACV2** was similar, except for the deacetoxymatricarin spot that was missing.

Isolation of Viscidulin A.—A portion of the **ACV1** sample (300 g) containing dried twigs and foilage was extracted with chloroform and processed in the usual manner.[5,21] The resulting dark sirup (15 g) was dissolved in a small amount of benzene and chromatographed on a 4 × 45 cm column of silica gel using benzene and benzene–diethyl ether of increasing polarity as eluents. One-hundred-milliliter aliquots were collected. The first ten aliquots of benzene eluted gums smelling of camphor and menthol. The following two fractions of the mixed solvent (95:5) gave a transparent gum which crystallized from ether–petroleum ether to give 15 mg of needles of viscidulin A (**1**): mp 124°; $[\alpha]_D$ +77.0° (c 2.14, $CHCl_3$); ir bands at 1773, 1733, 1243, and 1640 cm^{-1}; moderate uv end absorption; mass spectrum peaks at m/e 264 and 246; nmr data listed in Table I.

Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.67; H, 7.19. Found: C, 66.90; H, 7.03.

Viscidulin A was also obtained from the **ACV2** sample.

Isolation of Deactoxymatricarin.—The next two fractions (13 and 14) eluted from the above column with the same solvent mixture gave a transparent gum which crystallized from chloroform–ether and gave 40 mg of needles of deactoxymatricarin: mp 203–204° (alone or in admixture with an authetic sample);[21] ir bands at 1779 (γ-lactone), 1685 (cyclopentenone), 1639, and 1618 (unsaturation) cm^{-1}; nmr data listed in Table I.

Isolation of Viscidulin B.—The next few fractions (15 to 20) eluted with benzene–ether (9:1) showed a single purple spot on tlc and crystallized from chloroform–ether to give 140 mg of viscidulin B: mp 132–133°; $[\alpha]_D$ + 59.8° (c 2.3, $CHCl_3$); ir bands at 1779, 1735, 1254, 1647, and 885 cm^{-1}; λ_{max} 206 nm (ϵ 1050); mass spectrum peaks at m/e 306, 264, and 246; nmr data listed in Table I.

Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.67; H, 7.19. Found: C, 66.49; H, 7.24.

The same compound was also isolated from **ACV2**. Further elution of the column with more polar benzene–ether solvent mixtures (8:2, 7:3, and 6:4) and methanol gave colored gummy materials which could not be crystallized.

Isolation of Viscidulin C.—Extraction and processing of the **ACV2** sample (2.2 kg) as before gave 90 g of a sirup. Chromagraphy of this sirup on a 5 × 100 cm column of silica gel gave 2 g of viscidulin A, mp 124–125°, and 7.5 g of viscidulin B, mp 132–133°. Further elution of the column with more polar solvent mixtures (benzene–ether, 8:2, 7:3) gave a colorless gum which was crystallized from ether–petroleum ether to give 1.5 g of viscidulin C: mp 147°; $[\alpha]_D$ + 49.0° (c 1.955, $CHCl_3$); ir bands at 3496, 1745, 1639, and 836 cm^{-1}; uv end absorption; mass spectrum peaks at m/e 264 and 246; nmr data listed in Table I.

Anal. Calcd for $C_{15}H_{20}O_4$: C, 68.18; H, 7.57. Found: C, 68.39; H, 7.46.

The above compounds gave a positive color test for epoxide function.[11,12]

Hydrogenation of Viscidulin B.—A solution of 50 mg of viscidulin B in 15 ml of ethanol was stirred with 5% Pd/C catalyst in a hydrogen atmosphere for 2 hr, when it had absorbed 1.2 mol of hydrogen. The catalyst was then filtered and the filtrate was concentrated to a gummy product which showed four close tlc spots, but could not be resolved to separate compounds. The mixture had ir bands at 3510 (hydroxyl), 1779 (γ-lactone), 1735, and 1240 (acetate) cm^{-1}.

Reaction of Viscidulin B with Acetic Anhydride and *p*-Toluenesulfonic Acid.[16]—A solution of 40 mg of viscidulin B in 5 ml of acetic anhydride was refluxed with 30 mg of *p*-toluenesulfonic acid for 1.5 hr. The reaction gave a colored oil, which was chromatographed on a silica gel column. Elution of the column gave traces of a blue oil and dark gummy materials.

Reaction of Viscidulin B with *p*-Toluenesulfonic Acid and Acetic Acid.[7]—A solution of 1.2 g of viscidulin B in 20 ml of glacial acetic acid was cooled and treated with 400 mg of *p*-toluenesulfonic acid. The reaction mixture was kept in the refrigerator for 3 hr. It was then poured on crushed ice and extracted with chloroform. The chloroform extract was washed with sodium bicarbonate solution and distilled water. Removal of solvent left a colorless gum which showed four spots on tlc. Column chromatography of this gum gave lactones **6**, **7**, **8**, and **9** in pure form.

Lactone **6** was crystallized from ether–petroleum ether: yield 250 mg; mp 132°; ir bands at 1769, 1725, and 1220 cm^{-1}; λ_{max} 294 nm (ϵ 24.14); mass spectrum peaks at m/e 306, 264, 246, and 218; nmr spectrum listed in Table I.

Anal. Calcd for $C_{17}H_{22}O_5$: C, 66.67; H, 7.19. Found: C, 66.25; H, 7.29.

Lactone **7** was obtained as a chromatographically pure gel: yield 180 mg; ir bands at 3333, 1769, 1730, 1639, and 1235 cm^{-1}; mass spectrum peaks at 306, 246, and 231; nmr data listed in Table I.

Heating of this lactone formed a blue oil which condensed on the wall of the flask. The crude product was extracted with petroleum ether and purified by chromatography to give 12 mg of chamazulene as a deep blue oil. The pure oil on treatment with trinitrobenzene gave purple fibrous needles of the adduct, mp 132–133° (lit.[10,17] 132–133°).

Lactone **8** was crystallized from methanol as fine flakes: yield 96 mg; mp 192°; ir bands at 3571, 1770, 1730, 1628, and 1250 cm^{-1}; mass spectrum peaks at m/e 366, 324, 306, 288, 264, and 246; nmr data listed in Table I.

Anal. Calcd for $C_{19}H_{26}O_7$: C, 62.29; H, 7.10. Found: C, 62.63; H, 6.97.

Lactone **8** (40 mg) was treated with 5 ml of Tri-sil reagent in the usual manner. The resulting trimethylsilyl derivative was obtained as a solid which showed a single spot on tlc; ir bands at 1769 (γ-lactone), 1739 and 1245 (acetate), and 1639 (unsaturation) cm^{-1}; nmr spectrum listed in Table I.

(18) K. Tori, Y. Hamashima, and K. Takamizawa, *Chem. Pharm. Bull.*, **12**, 924 (1964).

(19) All melting points are uncorrected. The uv and ir spectra were recorded on Coleman–Hitachi EPS-3T and Beckman IR-5 spectrophotometers in 95% ethanol and in Nujol mulls, respectively. Mass spectra were determined on a Varian-Mat 111 spectrometer at 80 eV, using direct insertion. Baker A. R. No. 3405 silica gel was used for column chromatography and silica gel G Woelm was used for tlc. The plates were visualized by spraying with concentrated H_2SO_4 and heating.

(20) The samples were identified and collected by Professor M. S. Morris, School of Forestry, University of Montana.

(21) T. A. Geissman, T. Stewart, and M. A. Irwin, *Phytochemistry*, **6**, 901 (1967).

Lactone 9 was crystallized from chloroform–methanol as fine needles: yield 80 mg; mp 185°; ir bands at 3483, 3389, 1763, 1742, 1240, and 892 cm^{-1}; mass spectrum peaks at *m/e* 306, 264, and 246; nmr data listed in Table I.

Anal. Calcd for $C_{17}H_{24}O_6$: C, 62.96; H, 7.40. Found: C, 63.12; H, 7.31.

The monotrimethylsilyl derivative of lactone 9 was obtained in the manner described above. It gave a single spot on tlc and ir bands at 3521 (hydroxyl), 1769 (γ-lactone), 1739 and 1242 (acetate), and 1639 (unsaturation) cm^{-1}. The nmr data are listed in Table I.

Lactone 9 (35 mg) was dissolved in 2 ml of pyridine and 2 ml of acetic anhydride, and the solution was kept overnight at room temperature. Removal of the solvent gave lactone 9 monoacetate as a gum which resisted crystallization; the product gave a single spot on tlc and was chromatographically different from lactone 7; ir spectrum showed a broad band in the carbonyl region (γ-lactone and acetates) and other bands at 3500 (hydroxyl) and 1639 (unsaturation) cm^{-1}; nmr data are listed in Table I.

Deacetylation of Lactone 6 to Isoamberboin.—A solution of 60 mg of lactone 6 in 5 ml of 2% methanolic KOH was refluxed for 0.5 hr, acidified, and extracted with chloroform. Removal of the solvent left a colorless gum which was recrystallized twice to give 16 mg of isoamberboin as granules: mp 179–181° (lit.[8] 183°); $[\alpha]D$ +132° (*c* 1.026, $CHCl_3$); ir bands at 3475, 1750, 1737, and 1645 cm^{-1}.

Isolation of Cumambrin B.—Cumambrin B (2.5 g) and its acetate cumambrin A (0.9 g) were isolated in the usual manner from *Artemisia nova* Nels (450 g), collected near Red Rock, Mont. (T. 11 S, R. 10 W, Section 8), in August 1970. Cumambrin B had mp 178–179° (lit.[10] mp 178–180°); ir bands at 3509, 3290 (hydroxyl), 1750 (γ-lactone), and 1660 (unsaturation) cm^{-1}; nmr data listed in Table I.

Cumambrin A.—Cumambrin B (1.5 g) was acetylated with pyridine and acetic anhydride at room temperature to give 1.25 g of the crystalline monoacetate, cumambrin A: mp 188–189°, alone or in admixture with the naturally occurring cumambrin A isolated in the previous experiment (lit.[10] mp 188–190°); ir bands at 3533 (hydroxyl), 1745 (γ-lactone and acetate), 1248 (acetate), and 1663 (unsaturation) cm^{-1}; nmr data listed in Table I.

Dihydrocumambrin A.—A solution of 1.125 g of cumambrin A in 30 ml of ethyl acetate was hydrogenated in the presence of 280 mg of 5% Pd/C catalyst. Hydrogenation was stopped after 1 hr, when approximately 1 mol of hydrogen was absorbed. The catalyst was filtered off and the filtrate was evaporated to dryness. Crystallization of the residue from chloroform–ether gave 850 mg of dihydrocumambrin A as prisms: mp 171–172° (lit.[9] 170–173°); ir bands at 3510 (hydroxyl), 1782 (γ-lactone), 1748, and 1253 (acetate), cm^{-1}; nmr data listed in Table I.

Dehydration of Dihydrocumambrin A.—An ice-cold solution of 800 mg of dihydrocumambrin A in 8 ml of pyridine was treated with 2 ml of thionyl chloride and kept cold for 15 min. The reaction mixture was then lyophilized to remove the solvents and the residue obtained was dissolved in chloroform. The chloroform solution was filtered through a short column of silica gel. Evaporation of the colorless filtrate gave 675 mg of a transparent gum, which resisted crystallization. The product gave a single spot on tlc but the nmr data showed a mixture of exo and endo unsaturated lactones. Ir spectrum of the mixture had a broad band at 1786 to 1725 (γ-lactone and acetate), a sharp band at 1639 (unsaturation), and a broad band around 1250 (acetate) cm^{-1}.

Epoxidation of the Unsaturated Lactones.—A solution of 580 mg of the above mixture in 20 ml of chloroform was cooled to 0° and treated with an ice-cold solution of 415 mg of *m*-chloroperbenzoic acid in 10 ml of chloroform, and the reaction mixture was kept in the refrigerator. Progress of the reaction was monitored by periodic tlc of the reaction mixture for 24 hr until only traces of the starting material was left. The reaction mixture was then washed with $NaHCO_3$ solution and water. Removal of chloroform gave 490 mg of a colorless gum which gave several spots on tlc, including a spot corresponding to viscidulin B. Extensive chromatography of this mixture on silica gel gave viscidulin B, which was crystallized from chloroform–ether (yield 35 mg; mp 132–133°) alone or in admixture with the natural product. The ir and nmr spectra of these materials were also identical. Attempted isolation of other products from the reaction mixture was not successful.

Acetylation of Viscidulin C to Viscidulin B.—Viscidulin C (100 mg) was acetylated with pyridine and acetic anhydride at room temperature. Crystallization of the product from chloroform–ether gave viscidulin B (yield 95 mg; mp 132–133°) alone or in admixture with the natural product. The nmr and ir spectra of these materials were also identical.

Hydrogenation of Viscidulin A.—A solution of 468 mg of viscidulin A in 30 ml of ethyl acetate was hydrogenated in the presence of 125 mg of 10% Pd/C catalyst for 4 hr. The reaction gave a colorless product which resisted crystallization and showed four spots on tlc. Extensive chromatography of this material resulted in the isolation of lactones **14**, **15**, **16**, and **17**.

Lactone **14** was crystallized from ether–petroleum ether as needles: yield 75 mg; mp 156–157°; ir bands at 1770 (γ-lactone), 1736, and 1226 (acetate) cm^{-1}; mass spectrum peaks at *m/e* 232 and 217; nmr data listed in Table I.

Anal. Calcd for $C_{17}H_{24}O_4$: C, 69.86; H, 8.22. Found: C, 70.07; H, 8.45.

Lactone **15** (dihydroviscidulin A) gave fine needles after crystallization from ether–petroleum ether: yield 190 mg; mp 155–156°; ir bands at 1773 (γ-lactone), 1724, and 1234 (acetate) cm^{-1}; mass spectrum peaks at *m/e* 308 (M^+) and 248 (M − 60); nmr data listed in Table I.

Anal. Calcd for $C_{17}H_{24}O_5$: C, 66.23; H, 7.14. Found: C, 66.50; H, 7.91.

Lactone **16** was crystallized from ether–petroleum ether as fine needles: yield 3 mg; mp 142–144°; ir bands at 3500 (hydroxyl), 1760 (γ-lactone), 1730, and 1250 (acetate) cm^{-1}; mass spectrum peaks at *m/e* 250 and 232.

Lactone **17** was obtained in a small amount as a gum which showed a single spot on tlc but resisted crystallization. It had ir bands at 3450 (hydroxyl), 1760 (γ-lactone), 1725, and 1240 (acetate) cm^{-1}.

Reaction of Viscidulin A with *p*-Toluenesulfonic Acid in Acetic Acid.—A cold solution of 500 mg of viscidulin A in 20 ml of glacial acetic acid was treated with 200 mg of *p*-toluenesulfonic acid as before. The reaction gave a colorless gum which showed several spots on tlc but could not be fractionated or crystallized to pure compounds. The mixture had a strong ir band in the hydroxyl region.

Epoxidation of Viscidulin A.—A solution of 60 mg of viscidulin A in 10 ml of chloroform was treated with 60 mg of *m*-chloroperbenzoic acid and the reaction mixture was kept at room temperature for 3 hr. The mixture was washed with $NaHCO_3$ solution and the solvent was removed under reduced pressure. The product crystallized to give 45 mg of **18**: mp 142–143°; ir bands at 1755 (γ-lactone), 1720, and 1245 (acetate) cm^{-1}; mass spectrum peaks at *m/e* 322, 280, and 262; nmr data listed in Table I.

Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.35; H, 6.83. Found: C, 63.07; H, 6.67.

Epoxidation of Viscidulin B.—A solution of 200 mg of viscidulin B and 200 mg of *m*-chloroperbenzoic acid in 20 ml of chloroform was refluxed for 3 hr.[22] The gummy product showed two spots on tlc. Column chromatography of the mixture gave diepoxide **18** [yield 70 mg; mp 142–143° (alone or in admixture with the one obtained from viscidulin A)] and an epimeric diepoxide **19**: yield 41 mg; mp 148–149°; ir bands at 1760 (γ-lactone), 1710, and 1250 (acetate) cm^{-1}; mass spectrum peaks at *m/e* 322, 280, and 262; nmr data listed in Table I.

Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.35; H, 6.83. Found: C, 63.19; H, 7.01.

Registry No.—**1**, 35144-09-3; **2**, 35144-10-6; **3**, 35191-38-9; **4**, 17946-87-1; **6**, 23516-00-9; **6a**, 30825-69-5; **7**, 35144-13-9; **8**, 35144-14-0; **9**, 35191-40-3; **9a**, 35144-15-1; **9b**, 35144-16-2; **10**, 21982-83-2; **11**, 20482-33-1; **12**, 20482-39-7; **14**, 35144-20-8; **15**, 35144-21-9; **18**, 35191-41-4; **19**, 35191-42-5.

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